

Figure 1: Nucleotide sequences of the forward and reverse PCR primers used in the amplification of the genomic L-asparaginase sequences of *Wolinella succinogenes*.

[SEQ ID NO. 1] - Forward PCR Primer (BamHI Site Underlined)

5'-TCCGGATCCAGCGCCTCTGTTTTGATGGCT-3'

[SEQ ID NO. 2] - Reverse PCR Primer (EcoRI Site Underlined)

5'-TGGGAATTCGGTGGAGAAGATCTTTTGGAT-3'

0937982-032200

Figure 2: Agarose gel electrophoresis of propidium iodide-stained *Wolinella succinogenes* genomic DNA and a 1.0 Kb PCR fragment

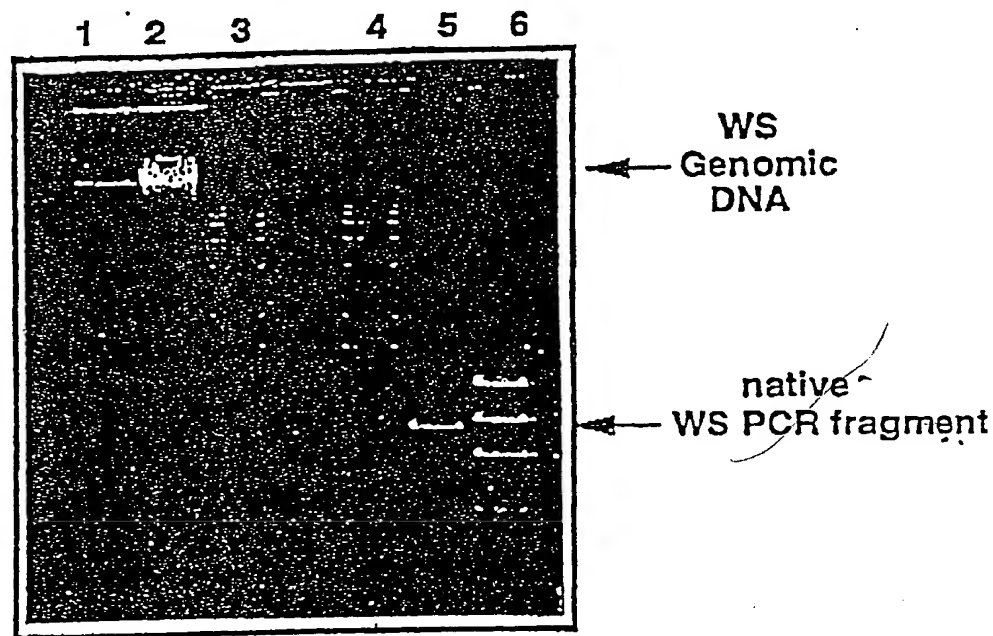


Figure 3: Restriction enzyme analysis of 4 colonies which were isolated following the ligation of the 1.0 Kb *Wolinella succinogenes*-specific PCR fragment into the PCR II vector

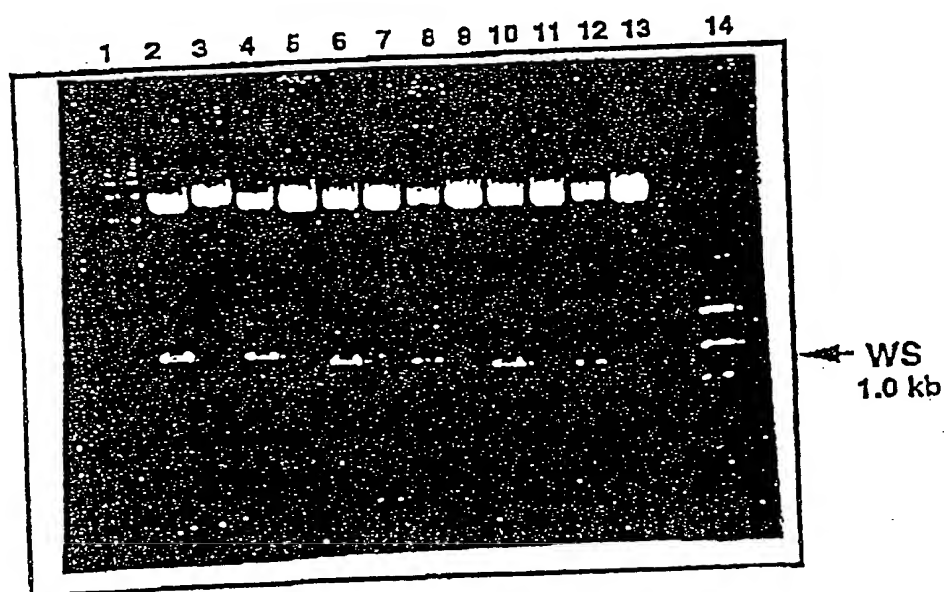
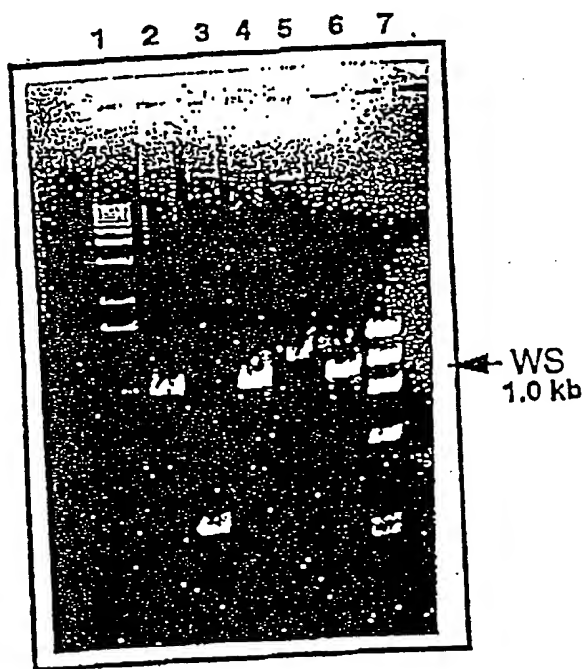


Figure 4: Agarose gel electrophoresis of the DNA fragments amplified from the selected, "positive" clones utilizing *Wolinella succinogenes* asparaginase-specific primers



09/937982

Figure 5: Determination of the anti-tumor activity of *Wolinella succinogenes* (WS), *Escherichia coli* (EC), and *Erwinia carotovora* (Erw) asparaginases against tumors generated by the injection of 6C3HED Gardener's lymphosarcoma cells in C3H mice

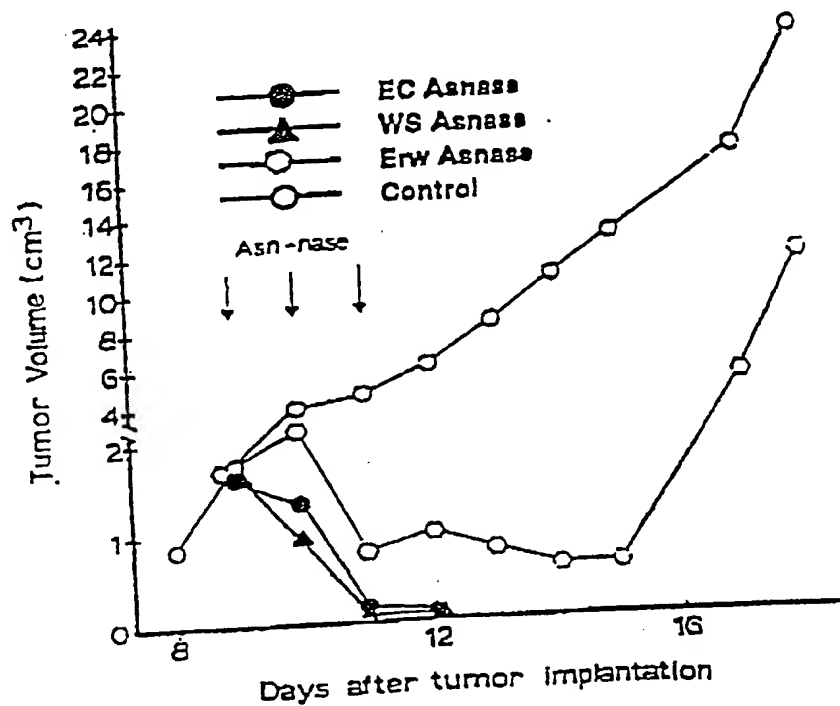
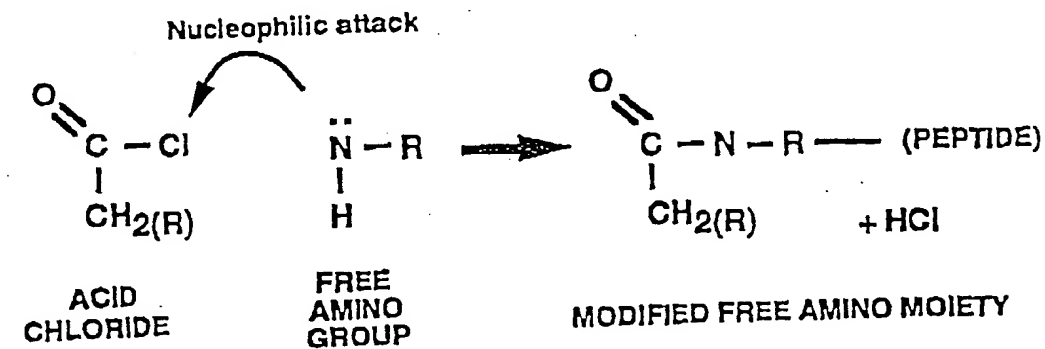


Figure 6: DNA sequence of the modified *Wolinella succinogenes* asparaginase-specific recombinant cDNA insert

ATG GGC AGC AGC **CAT** **CAT** **CAT** **CAT** **CAT** **CAT** AGC AGC GGC CTG GTG CCG
 CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT GGA CAG CAA ATG GGT CGC
 GGA TCC AGC GCC TCT GTT TTG ATG GCT AAA CCC CAA GTG ACT ATC CTA
 GCC ACA GGA GGC ACC ATC GCT GGT TCG GGG GAA TCT AGC GTC AAG AGT
 AGC TAC TCT GCT GGA GCA GTC ACC GTT GAT AAG CTT CTT GCA GCC GTC
 CCT GGC ATC AAC GAC CTA GCC ACC ATC AAG GGT GAA CAG ATC TCA AGC
 ATT GGC TCC CAA GAG ATG ACG GGT AAG GTG TGG CTT AAA CTA GCC AAG
 CGT GTC AAT GAG CTC CTC GCC CAA AAA GAG ACC GAA GCC GTG ATC ATC
 ACC CAT GGA ACT GAC ACC ATG GAA GAG ACC GCT TTC TTC CTC AAC CTC
 ACG GTG AAA AGC CAA AAA CCT GTC GTC CTT GTA GGC GCC ATG CGT CCA
 GGC TCT TCC ATG AGT GCT GAT GGC CCC ATG AAT CTC TAT AAC GCC GTG
 AAT GTA GCG ATC AAC AAA GCC TCT ACT AAC AAA GGA GTG GTG ATT GTG
 ATG AAC GAT GAG ATT CAC GCC GCC AGA GAA GCG ACC AAG CTC AAC ACC
 ACC GCA GTC AAT GCA TTT GCT TCG CCC AAC ACA GGT AAA ATC GGC ACA
 GTC TAT TAT GGC AAA GTC GAG TAT TTC ACT CAA TCC GTT CGA CCT CAC
 ACC CTT GCA AGT GAG TTT GAT ATT AGC AAA ATC GAA GAA CTC CCC AGA
 GTC GAT ATT CTT TAC GCT CAC CCC GAT GAT ACT GAT GTT TTA GTC AAT
 GCA GCC CTT CAG GCA GGA GCC AAA GGA ATC ATC CAT GCA GGC ATG GGC
 AAT GGG AAC CCT TTC CCT TTG ACT CAA AAT GCT CTT GAA AAA GCA GCC
 AAA TCA GGC GTA GTC GTC GCT CGA AGC TCT AGA GTG GGC AGT GGT TCC
 ACC ACC CAA GAG GCT GAA GTG GAT GAT AAG AAA CTT GGT TTT GTG GCT
 ACA GAG AGT CTC AAC CCT CAA AAA GCC AGA GTG CTT CTT ATG TTA GCC
 CTC ACC AAA ACT AGT GAT AGA GAG GCG ATC CAA AAG ATC TTC TCC ACC
 TAT **TAA** TCCAAGAAAGGGAATCTCTTCAC

The polyCAT sequence which encodes the polyHistidine residues, the ATG start site, and the TAA stop codon are shown in bold letters.

FIGURE 7



pH 8.5 to maintain protonated
state of nitrogen atom.

200006040600

FIGURE 8

Patient's Antibodies Against E.coli Asparaginase Do
Not Cross React With Wolinella Asparaginase

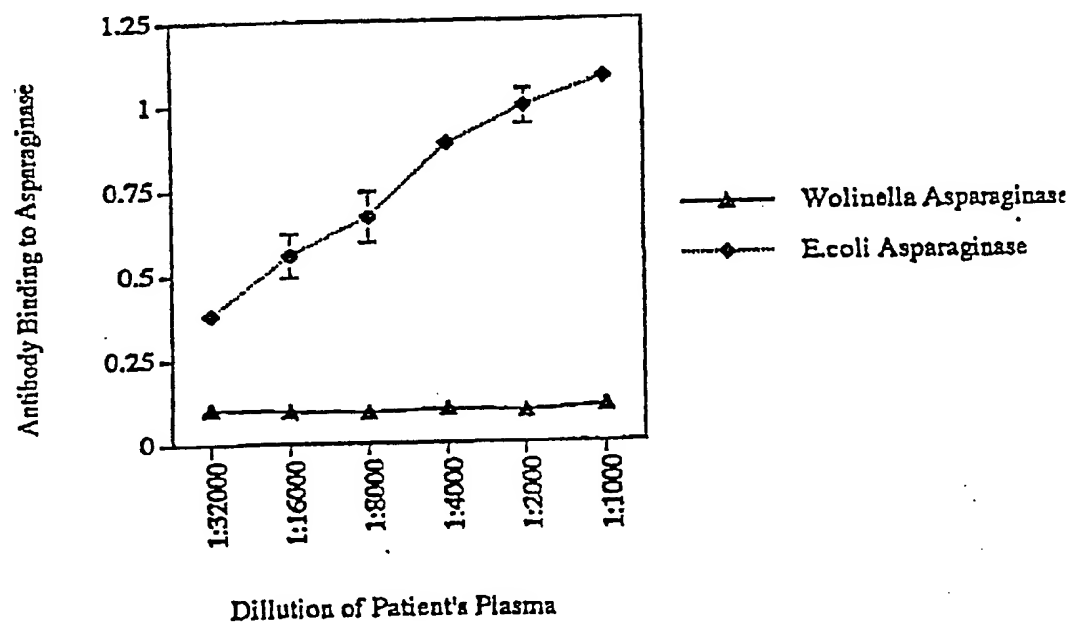


FIGURE 9

Binding of Asparaginase by Rabbit Anti-E.coli Asparaginase

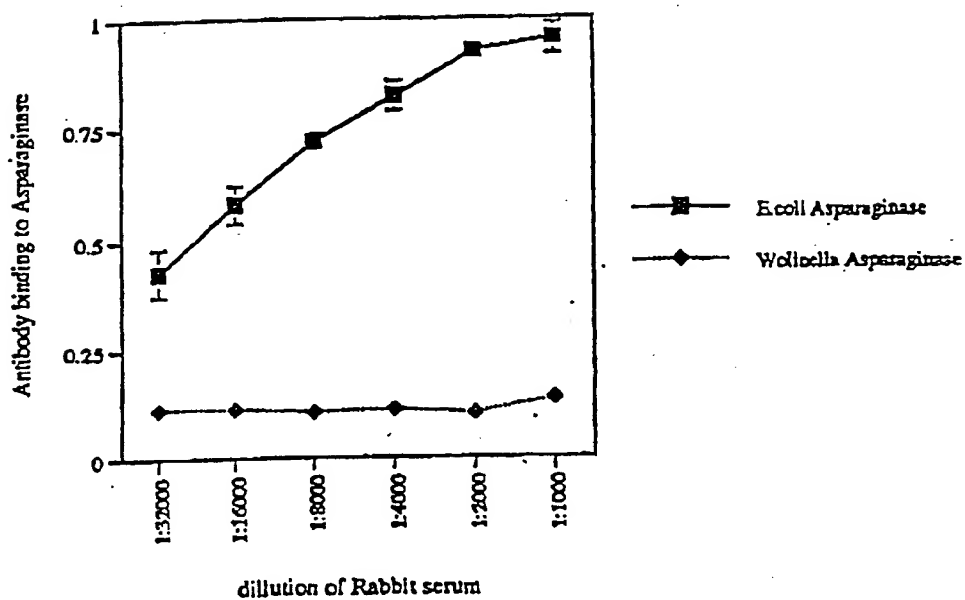
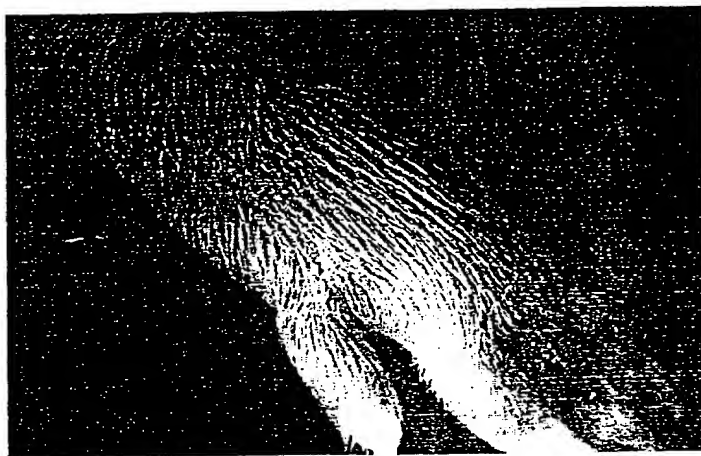


FIGURE 10



A



B

200000x 23.66um

Treatment of CIA DBA/1 Mice with E. coli L-Asparaginase
(Pilot Study)

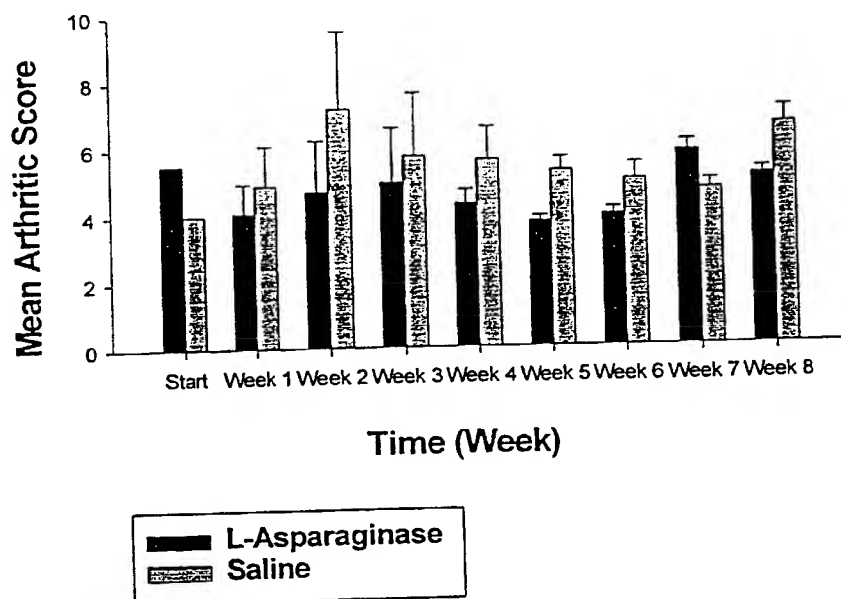


FIGURE 11

FIGURE 12

Mean Arthritic Score (MAS) for DBA/1 Mice
LPS Model

